Free PSA

Customer Support Center (USA)

1-800-527-1869

This package insert must be read carefully prior to use.
Package insert instructions must be followed accordingly.
Reliability of assay results cannot be guaranteed if there are deviations from the instructions in this package insert.

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, IL 60064

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List No. 3C20
Printed in U.S.A.

WARNING:
Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the AxSYM® Free PSA assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this assay package insert.

NAME
Free PSA (Prostate Specific Antigen)

INTENDED USE
The AxSYM® Free PSA assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative measurement of free prostate specific antigen (PSA) in human serum. The AxSYM Free PSA assay is intended to be used in conjunction with the AxSYM Total PSA assay in men aged 50 years or older with total PSA values between 4 and 10 ng/mL and non-suspicious DRE to determine the % free PSA value. The AxSYM % free PSA value can be used as an aid in discriminating between prostate cancer and benign disease.

SUMMARY AND EXPLANATION OF THE TEST
Prostate specific antigen (PSA), a member of the human kallikrein gene family, is a serine protease with chymotrypsin-like activity.[1-3] The mature form of PSA is a single chain glycoprotein of 237 amino acids containing 7-8% carbohydrate as a single N-linked oligosaccharide side chain. PSA has a molecular weight of approximately 30,000 daltons.[1,3,4]

The major site of PSA production is the glandular epithelium of the prostate. PSA produced by the prostate is secreted into the seminal fluid in high concentrations. PSA is also present in urine and serum.[3] The function of PSA is the proteolytic cleavage of gel forming proteins in the seminal fluid resulting in liquification of the seminal gel and increased sperm motility.[3,5] Low levels of PSA are found in the blood as a result of leakage of PSA from the prostate gland. Increasing levels of PSA are associated with prostatic pathology; including prostatitis, benign prostatic hyperplasia (BPH), and cancer of the prostate.[6-9]

PSA occurs in three major forms in the blood. The major immunodetectable form is PSA complexed with the serine protease inhibitor, alpha-1-antichymotrypsin (PSA-ACT). Uncomplexed, or free PSA, is the other immunodetectable form of PSA in serum. The majority of free PSA in serum appears to be an inactive form that cannot complex with protease inhibitors and may be either a PSA zymogen or an enzymatically-inactive, cleaved form of PSA. A third form of PSA, a complex with alpha-2-macroglobulin (AMG), is not detectable with current immunoassays for PSA due to the engulfment and subsequent masking of PSA epitopes by the alpha-2-macroglobulin molecule.[2,3,10]

Immunoassays have been designed to detect free PSA, PSA-ACT complex, and total PSA (immunodetectable forms: e.g. free PSA and PSA-ACT).[10-12] Using these types of assays, the proportion of free PSA in the serum was found to be significantly higher in patients with BPH than in patients with prostate cancer ($p<0.00001$).[12] The proportion, or percent, of free PSA determined by

comparing the concentration of free PSA to the concentration of total PSA has been proposed as a way to improve the discrimination between BPH and prostate cancer, especially in those men with intermediate levels of total serum PSA.[10,12-17]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM® Free PSA assay is based on Microparticle Enzyme Immunoassay (MEIA) technology. The AxSYM Free PSA Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

Sample and all AxSYM Free PSA reagents required for one test are pipetted by the Sampling Probe to various wells of the Reaction Vessel (RV).

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center with the Processing Probe.

PROCESSING CENTER

The Sample, Anti-PSA Coated Microparticles, and Assay Diluent are incubated together in one well of the reaction vessel. During the incubation of this reaction mixture the PSA in the specimen binds to the Anti-PSA Coated Microparticles forming an antibody-antigen complex.
An aliquot of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
The matrix cell is washed to remove unbound materials.
The Anti-Free PSA: Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds to the antibody-antigen complex.
The matrix cell is washed to remove unbound materials.
The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.

The concentration of free PSA in the sample is determined using a previously generated calibration curve.

For further information, refer to the AxSYM® System Operations Manual, Section 3.

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM Free PSA Reagent Pack (No. 3C20-20)*

1 Bottle (8.3 mL) Anti-PSA (Mouse, Monoclonal) Coated Microparticles in TRIS buffer with sucrose. Preservative: Sodium Azide. (Reagent Bottle 1)
1 Bottle (12.6 mL) Assay Diluent. TRIS buffer with protein (bovine) stabilizers. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent Bottle 2)
1 Bottle (16.0 mL) Anti-Free PSA (Mouse, Monoclonal): Alkaline Phosphatase Conjugate in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: Antimicrobial Agents. (Reagent Bottle 3)

1 Bottle (25.7 mL) Specimen Diluent. TRIS buffer with protein (bovine) stabilizers. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent Bottle 4)

* No. 3C20-66 includes an AxSYM Free PSA Reagent Pack (100 tests) plus Reaction Vessels (100 each) and matrix cells (100 each). No. 3C20-20 includes these items for international shipment.

CALIBRATORS

AxSYM Free PSA Master Calibrators (No. 3C20-30)

2 Bottles (4 mL each) of AxSYM Free PSA Master Calibrators contain PSA (human), donors of which have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg, prepared in TRIS buffer with protein (bovine) stabilizers to yield the following concentrations:

Bottle	Free PSA Concentration (ng/mL)*
1	0
2	2.5

Preservatives: Sodium Azide and Antimicrobial Agents.

Free PSA Calibrators (No. 9C12-01)

6 Bottles (4 mL each) of Free PSA Calibrators contain PSA (human), donors of which have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg, prepared in TRIS buffer with protein (bovine) stabilizers to yield the following concentrations:

Bottle	Free PSA Concentration (ng/mL)*
A	0
B	0.2
C	2.5
D	5
E	7.5
F	10

Preservatives: Sodium Azide and Antimicrobial Agents.

* The AxSYM Free PSA Master Calibrators and AxSYM Free PSA Calibrators are referenced against the Stanford 90:10 PSA Reference Material.[18-20]

CONTROLS

Free PSA Controls (No. 9C12-10)

3 Bottles (8 mL each) of Free PSA Controls contain PSA (human), donors of which have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg, prepared in TRIS buffer with protein (bovine) stabilizers to yield the following concentration ranges:

	Free PSA	
Bottle	Concentration (ng/mL)	Range (ng/mL)
L	0.4	0.3 - 0.5
M	1	0.8 - 1.2
H	7	5.6 - 8.4

Preservatives: Sodium Azide and Antimicrobial Agents.

The AxSYM® Free PSA default result unit is ng/mL. An alternate unit (µg/L) may be selected for reporting results (Assay Parameter 45). The conversion formula to change the alternate unit is: ng/mL x 1.0 = µg/L.

SPECIMEN DILUENT

Free and Total PSA Specimen Diluent (No. 9C46-50)

1 Bottle (100 mL) TRIS buffer with protein (bovine) stabilizers. Preservatives: Sodium Azide and Antimicrobial Agents.

OTHER REAGENTS

AxSYM Probe Cleaning Solution (No. 9A35-05)

2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (TEAH).

Solution 1 (MUP) (No. 8A47-04)

4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: Sodium Azide.

Solution 3 (Matrix Cell Wash) (No. 8A81-04)

4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

Solution 4 (Line Diluent) (No. 8A46)

1 bottle (10 L) Solution 4 (Line Diluent) containing 0.1M Phosphate Buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only.

SAFETY PRECAUTIONS

This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **REAGENTS** section of this package insert. Donors of human sourced materials have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[21] Biosafety Level 2[22] or other appropriate biosafety practices[23,24] should be used for materials that contain or are suspected of containing infectious agents.

The AxSYM® Probe Cleaning Solution (2% TEAH) may cause mild skin or eye irritation. If this solution comes in contact with skin or eyes, flush immediately with water.

Some components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Economic Community (EEC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R22 Harmful if swallowed. R32 Contact with acids liberates very toxic gas.

S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36 Wear suitable protective clothing.
S46 If swallowed, seek medical advice immediately and show this container or label.

HANDLING PRECAUTIONS

Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on-board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.

Do not use Reagent Pack beyond the expiration date or a maximum of 336 cumulative hours on-board the AxSYM system.

Do not mix reagents from different reagent packs.

Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS

The AxSYM® Free PSA Reagent Pack and Master Calibrators, the Free PSA Calibrators and Controls, and the Free and Total PSA Specimen Diluent must be stored at 2-8°C (do not freeze). The Reagent Pack, Master Calibrators, Calibrators, Controls, and Specimen Diluent may be used immediately after removing them from the refrigerator. Master Calibrators, Calibrators, Controls, and Specimen Diluent should be returned to 2-8°C storage immediately after use.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Free PSA Reagent Pack may be on–board the AxSYM System for a maximum of 336 cumulative hours; for example, 42 eight hour shifts. Recalibration may be required to obtain maximum on-board reagent stability. More frequent use of controls may be required to monitor reagent performance within the same lot. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendices, for further information on tracking on–board time.

Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removing it from the refrigerator. MUP may be on-board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded.

The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash) and Solution 4 (Line Diluent) must be stored at 15-30°C.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM Free PSA assay file must be installed on the AxSYM System from the AxSYM Cancer Assay Disk (List No. 3D50-01 or higher) prior to performing Free PSA assays. This assay disk contains a PSA_Ratio file which may also be installed. This PSA_Ratio file allows the AxSYM instrument to calculate the ratio of the AxSYM Free PSA concentration to the AxSYM Total PSA concentration (List No. 3C19). Refer to the AxSYM System Operations Manual, Section 2: Installation Procedures and Special Requirements, for proper installation procedures.

AxSYM Free PSA ASSAY PARAMETERS

The default values for the assay parameters used for the AxSYM Free PSA assay are listed below. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System

Operations Manual, Section 2: Installation Procedures and Special Requirements. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen. Press PRINT to print the assay parameters.

Assay Parameters

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1 Long Assay Name (English): PSA_Free
6 Abbrev Assay Name (English): PSA_Free

11 Assay Number: 442
12 Assay Version: *
13 Calibration Version: *
14 Assay File Revision: *
15 Assay Enabled > ON
17 Assay Type: MEIA
18 Standard Cal Reps > 2
19 Master Cal Reps > 2
21 Cal A Concentration: 0.000
22 Cal B Concentration: 0.200
23 Cal C Concentration: 2.500
24 Cal D Concentration: 5.000
25 Cal E Concentration: 7.500
26 Cal F Concentration: 10.000
27 Master Calibrator 1 Concentration: 0.000
28 Master Calibrator 2 Concentration: 2.500
43 Default Dilution Protocol > UNDILUTED
44 Default Calibration Method > Standard Cal
45 Selected Result Concentration Units > ng/mL
46 Selected Result Decimal Places > 3
91 Low Range Undiluted: 0.000
92 High Range Undiluted: 10.000
96 Low Range Dil 1: 2.000
97 High Range Dil 1: 100.000

NOTE: Parameter 45 can be edited to the alternate result unit µg/L.

Refer to the AxSYM® System Operations Manual for a detailed description of Instrument Procedures.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Human serum only (including serum collected in serum separator tubes) may be used in the AxSYM® Free PSA assay. Follow the manufacturer's processing instructions for serum collection tubes.

It is recommended to obtain specimens for PSA testing prior to procedures involving manipulation of the prostate. Refer to the LIMITATIONS OF THE PROCEDURE section in this assay package insert.

The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type is used in the AxSYM Free PSA assay. Ensure that complete clot formation has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.

Do not test grossly hemolyzed specimens.

Serum should be separated from the clot within 3 hours from time of collection and stored at 2-8°C. The serum, if not tested within 24 hours, should be frozen (-20°C or colder).[25,26]

Multiple freeze-thaw cycles should be avoided. Specimens must be mixed **THOROUGHLY** after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use, to remove particulate matter and to ensure consistency in the results.

Free PSA Calibrators and Controls should be mixed by gentle inversion prior to use.
Patient specimens should be mixed and centrifuged after any freeze-thaw cycle or to remove red blood cells or particulate matter.

To minimize the effects of evaporation, all samples (patient specimens, controls and calibrators) should be tested within 3 hours of being placed on-board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5: Operating Instructions, Subsection: Inventory and Loading Consumables, for a more detailed discussion of on-board sample storage constraints.

Inspect all samples for bubbles. Remove bubbles prior to analysis.

Performance has not been established using body fluids other than human serum.

Specimens with obvious microbial contamination should not be used.

When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical samples and infectious substances.

SAMPLE VOLUME

The sample volume required to perform a single undiluted Free PSA test on the AxSYM® System varies depending on the type of sample container used. For sample cups, a ROUTINE or STAT sample test requires 167 µL. For every additional Free PSA test performed (ROUTINE or STAT) from the same sample container, an additional 117 µL of sample is required.

The sample cup minimum volume for both ROUTINE and STAT tests is calculated by the AxSYM System. It is displayed on the Order screen at the time the test(s) is(are) ordered and printed on the Orderlist Report. When using Host Order Query, the Order screen information and the Orderlist Report are not available. Refer to the AxSYM Systems Operations Manual, Section 5: Operating Instructions, Subsection: Ordering Patient Samples, for a description of the Host Order Query option.

If the assay is configured for auto retest or reflex testing, the additional sample volume needed for the retest will not be displayed on the order screen at the time the test(s) is (are) ordered. Therefore, the total sample volume should include an additional 117 µL of sample.

To obtain the recommended volume requirements for the Free PSA Calibrators and Controls, hold the bottles **vertically** and dispense 8 drops of each Calibrator or 5 drops of each Control into each respective sample cup. Refer to the AxSYM System Operations Manual, Section 5: Operating Instruction, Subsection: Loading Samples, Cals, and Controls, for sample volume requirements in primary or aliquot tubes and calibrator/control requirements for multiple reagent lots.

AxSYM FREE PSA PROCEDURE

Materials Provided

No. 3C20-66	AxSYM Free PSA Reagent Kit, containing: AxSYM Free PSA Reagent Pack 100 reaction vessels 100 matrix cells

Materials Required But Not Provided

AxSYM® System

No. 9C12-10	Free PSA Controls
No. 9C12-01	Free PSA Calibrators
or	
No. 3C20-30	AxSYM Free PSA Master Calibrators
No. 9C46-50	Free and Total PSA Specimen Diluent
No. 8A47-04	Solution 1 (MUP)
No. 8A81-04	Solution 3 (Matrix Cell Wash)
No. 8A46	Solution 4 (Line Diluent)
No. 9A35-05	AxSYM Probe Cleaning Solution
No. 8A76-01	Sample cups

Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen

CAUTION:

When manually dispensing sample into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use accurately calibrated equipment.

For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9: Service and Maintenance. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

Sections 5 and 6 of the AxSYM System Operations Manual can be easily removed for use at the instrument. They contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of matrix cells, bulk solutions, and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup volume requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments. When using Host Order Query, the Orderlist Report is not available. Refer to the AxSYM® System Operations Manual, Section 5: Ordering Patient Samples, for a description of the Host Order Query Option.

CAUTION: When operating the AxSYM System, always observe the following:
The System status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, reagent packs, or reaction vessels (RVs).

Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. Tests in process will be terminated and must be repeated.

When testing is completed, it is recommended that samples and the AxSYM Free PSA Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store at 2-8°C.

SAMPLE DILUTION PROCEDURES

Patient specimens with a Free PSA value exceeding 10 ng/mL (HIGH RANGE, assay parameter 92), are flagged with the code >"10" or associated error code(s). To quantitate the concentration of these specimens, perform either the Automated Dilution Protocol or the Manual Dilution Protocol.

Automated Dilution Protocol

The Automated Dilution Protocol is provided to assist in quantitating test results greater than 10 ng/mL up to 100 ng/mL. The AxSYM System performs a 1:10 dilution of the unknown samples using one reaction vessel. The AxSYM System automatically calculates the concentration of the diluted sample and reports the result.

If the assay is configured for auto dilution, the additional 55 µL of sample volume needed for the dilution should be included in the sample container when ordering tests. Refer to Section 5 of the AxSYM System Operations Manual for additional information on ordering sample dilutions.

Manual Dilution Protocol

An automated or manual dilution can be performed for samples with free PSA concentrations >10 to 100 ng/mL. Patient specimens with free PSA concentrations of >10 ng/mL can be diluted using a

suggested manual dilution of 1:10. Add 100 µL of the patient specimen to 900 µL of Free and Total PSA Specimen Diluent (No. 9C46-50). A manual dilution must be performed on all samples >100 ng/mL. The dilution should be performed so that the diluted test results read greater than the Free PSA Calibrator B (0.2 ng/mL) on the calibration curve. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

Final Sample Concentration = Reported Concentration x Manual Dilution Factor

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Sample + Volume of Dilution Reagent)}}{\text{Volume of Sample}}$$

Automatic Sample Retesting

Patient specimens with PSA concentrations falling within a user specified range may be automatically retested by the instrument for the same assay or a different assay. For example, if a total PSA concentration falls within the specified range set by the user, the specimen can automatically be retested by the assay chosen as the retest assay (e.g. Free PSA) without user intervention or the need to reorder the test. Ensure that the additional sample volume needed for the retest assay (117 µL for the AxSYM Free PSA assay) is added to the sample container when ordering the tests.

An automated 1:10 dilution protocol can be selected for any specimen with a Free PSA concentration greater than 10 ng/mL. If the assay is configured for auto dilution, ensure that the sample volume needed (55 µL for the AxSYM Free PSA assay) is added to the sample container when ordering the test. To implement automated sample retesting, the user must define the appropriate retest rule(s). Refer to Section 2 of the AxSYM System Operations Manual for detailed instructions on Automatic Sample Retesting.

Calculation of AxSYM % free PSA Value

The AxSYM system can automatically calculate the PSA Ratio when both AxSYM Free PSA and AxSYM Total PSA assays are ordered on the same sample. The PSA Ratio is calculated and printed after the Free PSA and Total PSA results for the specific sample are printed. The PSA ratio can be converted to the AxSYM % free PSA value by multiplying the ratio value X 100. Follow the steps below to configure the AxSYM System for the PSA Ratio calculation.

Ensure that AxSYM Free PSA, AxSYM Total PSA, and PSA_Ratio files are all installed properly. The PSA_Ratio file is located on the AxSYM Free PSA assay disk. Refer to Section 2 of the AxSYM System Operations Manual for the Assay Installation procedure.

Ensure that active assay calibration curves are generated and stored on the system for both AxSYM Free PSA and AxSYM Total PSA assays.
Enable the PSA Ratio calculation by selecting the formula displayed on the Configuration/Ratio screen as shown below:

PSA_Free/PSA_Total

Note: Select "Ratio Disabled" to turn off the function of Ratio calculation.

Press F6-SAVE to update the system with the selection.

Once the PSA Ratio is configured, the AxSYM Free PSA and AxSYM Total PSA assays must be ordered at the same time on each sample to obtain the ratio result for that sample. A PSA Ratio will also be generated, if a retest rule is used in the AxSYM Total PSA Assay to retest with the AxSYM Free PSA assay. After the Free PSA and Total PSA assays are completed, and the results are printed, the PSA Ratio value will automatically be calculated and printed. Multiply the PSA Ratio value by 100 to obtain the AxSYM % free PSA value. Refer to Section 2 of the AxSYM System Operations Manual for detailed instructions on Ratio Configuration.

QUALITY CONTROL PROCEDURES

CALIBRATION

The AxSYM Free PSA assay must be calibrated using either a Master Calibration (2-point), or a Standard Calibration (6–point) procedure. The use of a particular calibration procedure is dependent on individual laboratory policy.

Master Calibration

Each AxSYM Free PSA Reagent Pack is shipped with a bar code that contains the Master Curve for that specific lot of reagents. When using a new lot number for the first time, the Master Curve must be entered into the AxSYM System. Refer to the AxSYM System Operations Manual, Section 6: Calibration Procedures, Subsection: Calibration Orders, for additional information on entering Master Curve bar codes. Once this bar code information is entered, a Master Calibration must be performed.

To perform an AxSYM Free PSA Master Calibration, test Master Calibrators 1 and 2 in duplicate. A single sample of all levels of Free PSA controls must be tested as a means of evaluating the assay calibration.

Standard Calibration

The Standard Calibration procedure may be used without prior entry of the bar coded Master Curve information. To perform an AxSYM® Free PSA Standard Calibration, test Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of Free PSA controls must be tested as a means of evaluating the assay calibration.

Once the AxSYM Free PSA calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

A reagent pack with a new lot number is used.
Controls are out of range.
The MEIA Optics Verification Update has been performed.

Refer to the AxSYM System Operations Manual Section 6 for:

Setting up an assay calibration
When recalibration may be necessary

Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10: Troubleshooting and Diagnostics, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The recommended control requirement for an AxSYM Free PSA assay is a single sample of all Free PSA control levels tested once every 24 hours each day of use. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

Ensure that assay control values are within the concentration ranges specified in the package insert. Refer to the **REAGENTS**, CONTROLS section of this package insert for Free PSA Control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a Free PSA control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require retesting. Assay recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, Subsection: Observed Problems, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

Fluorescence Background Acceptance Criteria

Quality Control with regards to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64 Max Intercept-Max MUP intercept each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10: Troubleshooting and Diagnostics, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2: Installation Procedures and Special Requirements, for further information on this parameter.

RESULTS

CALCULATION

The AxSYM Free PSA assay utilizes a 4-parameter logistic data reduction method (4PLC) to generate a Standard Calibration curve. The Master Calibration uses a linear transformation (LT) technique to adjust the Master Curve. Refer to the AxSYM System Operation Manual, Appendix F, for further information.

The AxSYM % free PSA value can be calculated by multiplying the PSA Ratio from the data printout by 100.

ALTERNATE RESULT UNIT

The default result unit for AxSYM Free PSA is ng/mL. When selecting the alternate result unit, µg/L, the conversion factor used by the AxSYM System is 1.0.

The conversion formula to change to the alternate unit is: ng/mL x 1.0 = µg/L

FLAGS

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2: Installation Procedures and Special Requirements.

LIMITATIONS OF THE PROCEDURE

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[27,28] These specimens should not be assayed with the AxSYM Free PSA assay.

Heterophilic antibodies in serum have the potential to cause interference in immunoassay systems.[29,30] Infrequently, PSA levels may appear elevated due to heterophilic antibodies present in the patient's serum or to nonspecific protein binding. If the PSA level is inconsistent with clinical evidence, additional PSA testing is suggested to confirm the result.

The concentration of PSA in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.[3,31,32]

Quality control samples may be produced by introducing seminal fluid PSA into a human serum matrix. PSA in serum and seminal fluid may exist in different forms. The concentration of PSA in these controls, determined with assays from different manufacturers, can vary due to

differences in assay methods, calibration, reagent specificity, and the form of PSA that is present; therefore, it is important to use assay-specific values to evaluate control results.

Digital rectal examination (DRE) may cause clinically significant changes in the free PSA and free/total PSA ratio in some patients.[33] Additionally, prostatic massage, ultrasonography, cystoscopy, and needle biopsy may cause clinically significant elevations.[33,34] Serum for free PSA determinations should be drawn before performing prostatic manipulations. PSA levels may also be increased following ejaculation.[35]

Active free PSA in the serum at the time of blood sampling, can continue to complex with serum protease inhibitors, especially alpha-2-macroglobulin, resulting in a rapid decrease in PSA levels of the active form of free PSA.[36]
Hormonal therapy may affect PSA expression, therefore, a low PSA level after any treatment that includes hormonal therapy may not adequately reflect the presence of residual or recurrent disease.[37]

The measurement of free PSA or the % free PSA value is not an absolute test for malignancy. The PSA values should be used in conjunction with information available from the clinical evaluation and other diagnostic procedures: e.g. symptoms, clinical impressions, digital rectal examination, transrectal ultrasound, etc. A prostatic biopsy is required for the diagnosis of cancer.

Refer to the **SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert.

EXPECTED VALUES

The distribution of AxSYM Free PSA values determined in apparently healthy males, males with BPH, and males with confirmed prostate cancer is shown below.

Distribution of AxSYM Free PSA Values

	Number of Subjects	Percent (%) 0-0.934 (ng/mL)	>0.934-2.5 (ng/mL)	>2.5-5.0 (ng/mL)	>5.0-10 (ng/mL)	>10 (ng/mL)
Healthy Males	999	95.1	4.5	0.3	0.1	0.0
BPH	368	73.6	18.2	5.4	1.4	1.4
Prostate Cancer	498	60.0	21.9	6.2	4.8	7.0

This distribution table is derived from 999 healthy male subjects (250 <50 years of age and 749 ≥50 years of age) with no clinical evidence of prostate cancer, 368 males with BPH and 498 males with active prostate cancer. In this study, 95% of the specimens from apparently healthy males had free PSA values of 0.934 ng/mL or less.

The total PSA values were determined using the AxSYM Total PSA assay for the 1,865 subjects in the table above. The % free PSA value has been proposed as a way to improve the discrimination between BPH and prostate cancer, especially in those men with intermediate levels of total PSA.[10,12-17] The AxSYM % free PSA value was determined for the 621 subjects with total PSA values between 2-20 ng/mL. The distribution of those values is shown in the following table.

Distribution of AxSYM % free PSA Values for Specimens with AxSYM Total PSA Between 2 and 20 ng/mL

Distribution of Subjects (%)						
	Number of Subjects	% Free PSA Ranges				
		≤10	>10-15	>15-20	>20-26	>26
Healthy Males	170	8.8	15.3	21.8	26.5	27.6
BPH	176	10.2	15.3	21.6	20.5	32.4
Prostate Cancer	275	38.2	25.8	17.1	9.5	9.5

An additional prospective study of 271 subjects (181 biopsy negative and 90 biopsy positive) was conducted at nine clinical sites across the United States. Cutoffs for % free PSA were identified that would yield a sensitivity of at least 90% or 95%, and a specificity level of at least 90% or 95% for subjects with a total PSA range of 4 to 10 ng/mL and a DRE non-suspicious for cancer. The total and free PSA values were determined using the AxSYM Free PSA and AxSYM Total PSA assays. The following table shows the % free PSA cutoffs at or above 90%, and 95% sensitivity and specificity.

Percent Free PSA Cutoffs at or Above 90% and 95%

Sensitivity and Specificity

% Free PSA	Sensitivity (%)	95% CI	Specificity (%)	95% CI
9.7	24.4	(16.0,34.6)	95.0	(90.8,97.7)
10.9	32.2	(22.8,42.9)	90.1	(84.7,94.0)
23.6	90.0	(81.9,95.3)	40.3	(33.1,47.9)

26.4	96.7	(90.6,99.3)	28.2	(21.8,35.3)

The distribution of AxSYM % free PSA values was determined for 271 subjects with total PSA values between 4 and10 ng/mL and DRE non-suspicious for cancer. The % free PSA values were divided into five groups by the following boundaries: ≤ 10, > 10-15, > 15-20, > 20-26 and >26. The table below shows the % free PSA values for 271 subjects (181 biopsy negative and 90 biopsy positive).

Distribution of AxSYM % free PSA Values

for Specimens with AxSYM Total PSA

Between 4 and 10 ng/mL

Distribution of Subjects						
	% Free PSA Ranges					
	Number of Subjects	≤10	>10-15	>15-20	>20-26	>26
Biopsy Negative	181	6.1□	16.0	24.9	24.9	28.2
Biopsy Positive	90	30.0	28.9	22.2	13.3	5.6

The probabilities of prostate cancer given the value in specific ranges for % free PSA were calculated based on a logistic regression model using the same group of subjects as above. Prostate cancer probabilities associated with % free PSA values are dependent on the disease prevalence within the study population. [38] In this study, the probabilities of prostate cancer are representative of a patient population for both screening and referral sites with an overall disease prevalence of approximately 33%.[39] The table below shows the distribution of cancer probabilities of % free PSA using the same study population adjusted for different rates of disease prevalence.

Probability of Prostate Cancer by Disease Prevalence For Subjects with AxSYM Total PSA between 4 and 10 ng/mL and DRE Non-suspicious for Cancer.

Disease Prevalence	% Free PSA Ranges				
	≤ 10	> 10- 15	> 15 - 20	>20 - 26	>26
25 [40]	58.7	40.2	24.1	13.0	6.6
33	**67.9**	**50.0**	**32.1**	**18.3**	**9.6**
35	69.6	52.0	33.9	19.5	10.3
45	77.7	62.2	43.8	26.9	14.8

The estimates of cancer probability may be influenced by the presence of other risk factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Precision was determined as described in the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-T2.[41] Six samples consisting of 3 serum based panels and 3 Free PSA Controls were assayed at 4 laboratories (N=80 for each sample) in replicates of 2 at two separate times per day for twenty days using a single lot of reagents and a single calibration. Data from this study are summarized below.

Reproducibility of AxSYM Free PSA

	Lab	Mean Free PSA (ng/mL)	Within Run (%)CV	Total (%) CV
Low Control	1	0.4	4.81	5.42
	2	0.38	2.37	3.13
	3	0.36	2.38	4.36
	4	0.39	2.62	6.27
Medium Control	1	1.01	4.44	5.5
	2	0.97	2.53	3.18
	3	0.92	3.9	5.19
	4	0.98	2.15	7.04
High Control	1	7.34	5.18	6.73
	2	6.92	3.15	3.32
	3	6.48	3.35	5.07
	4	7.25	2.98	8.24
Panel 1	1	0.43	4.32	4.63
	2	0.41	3.01	3.46
	3	0.38	3.12	4.23
	4	0.41	3	6.58
Panel 2	1	3.68	4.46	5.55
	2	3.6	3.09	3.53
	3	3.37	2.78	4.12
	4	3.63	2.66	7.25
Panel 3	1	6.53	5	6.16
	2	6.35	3.23	3.66
	3	5.95	3.44	4.17
	4	6.45	3.14	7.82

The standard deviation may be calculated by multiplying the mean PSA concentration by the percent CV and dividing by 100.

$$SD = \frac{\text{Mean (ng/mL)} \times \text{(\%)CV}}{100}$$

RECOVERY

Known concentrations of serum PSA were added to normal human serum samples. The concentration of PSA was determined using the AxSYM Free PSA assay and the resulting percent recovery was calculated.

Recovery of PSA

Sample Type	Endogenous Level (ng/mL)	PSA Added (ng/mL)	Value Obtained (ng/mL)	(%) Percent Recovery
NORMAL HUMAN	0	0.96	0.98	102
SERUM		5.55	5.44	98
	0.08	0.98	1.00	94
		5.80	5.45	93
	0.3	0.96	1.25	99
		5.55	5.68	97

$$\% \text{ Recovery} = \frac{\text{PSA Value Obtained (ng/mL)} - \text{Endogenous Level (ng/mL)}}{\text{PSA Added (ng/mL)}} \times 100$$

ANALYTICAL SENSITIVITY

The sensitivity of the AxSYM Free PSA assay was calculated to be better than 0.02 ng/mL. This sensitivity is defined as the concentration at two standard deviations above the Free PSA Calibrator A (0 ng/mL) and represents the lowest measurable concentration of PSA that can be distinguished from zero.

ANALYTICAL SPECIFICITY

The specificity of the AxSYM Free PSA assay was determined by testing sera containing the compounds listed below. These compounds did not show interference in the AxSYM Free PSA assay at the levels indicated.

INTERFERING SUBSTANCES

Test Compound	Test Concentration	
Bilirubin	50	mg/dL
Hemoglobin	1,000	mg/dL
IgG	3,000	mg/dL
Total Protein	3-14	g/dL
Triglcerides	3,000	mg/dL
Prostatic Acid Phosphatase (PAP)	1,000	ng/mL

CHEMOTHERAPEUTIC AGENTS

Test Compound	Test Concentration	
Cyclophosphamide	700	µg/mL
Diethylstilbestrol	2	µg/mL
Doxorubicin-HCl	16	µg/mL
Estramustine Phosphate	200	µg/mL
Flutamide	10	µg/mL
Goserelin Acetate	100	ng/mL
Lupron®	100	µg/mL
Megestrol Acetate	90	µg/mL
Methotrexate	30	µg/mL
Proscar®	25	µg/mL
Hytrin®	10	µg/mL

CROSS REACTIVITY

The cross reactivity of the AxSYM Free PSA Assay with PSA-ACT measured at PSA-ACT concentrations up to 106 ng/mL was less than 1%.

CARRYOVER

No significant carryover (less than 2.5 ppm) was detected when a sample containing 17,115 ng/mL of PSA was assayed.

BIBLIOGRAPHY

1. Watt KWK, Lee P-J, Timkulu TM, *et al.* Human Prostate-Specific Antigen: Structural and Functional Similarity with Serine Proteases. *Proc Natl Acad Sci USA* 1986;83:3166–70.

2. Christensson A, Laurell C-B, Lilja, H. Enzymatic Activity of Prostate-Specific Antigen and its Reactions with Extracellular Serine Proteinase Inhibitors. *Eur J Biochem* 1990;194:755–63.

3. McCormack RT, Rittenhouse HG, Finlay JA, *et al.* Molecular Forms of Prostate-Specific Antigen and the Human Kallikrein Gene Family: A New Era. *Urology* 1995;45:729–44.

4. Bélanger A, van Halbeek H, Graves HCB, *et al.* Molecular Mass and Carbohydrate Structure of Prostate Specific Antigen: Studies for Establishment of an International PSA Standard. *Prostate* 1995;27:187–97.

5. Lilja H. A Kallikrein-Like Serine Protease in Prostatic Fluid Cleaves the Predominant Seminal Vesicle Protein. *J Clin Invest* 1985;76:1899–903.

6. Kuriyama M, Wang MC, Papsidero LD, *et al.* Quantitation of Prostate-Specific Antigen in Serum by a Sensitive Enzyme Immunoassay. *Cancer Res* 1980;40:4658–62.

7. Stamey TA, Yang N, Hay AR, *et al.* Prostate-Specific Antigen as a Serum Marker for Adenocarcinoma of the Prostate. *New Engl J Med* 1987;317:909–16.

8. Oesterling JE. Prostate Specific Antigen: A Critical Assessment of the Most Useful Tumor Marker for Adenocarcinoma of the Prostate. *J Urol* 1991;145:907–23.

9. Partin AW and Oesterling JE. The Clinical Usefulness of Prostate Specific Antigen: Update 1994. *J Urol* 1994;152:1358–68.

10. Stenman U-H, Leinonen, J, Alfthan H, *et al.* A Complex Between Prostate-Specific Antigen and α1-Antichymotrypsin is the Major Form of Prostate-Specific Antigen in Serum of Patients with Prostatic Cancer: Assay of the Complex Improves Clinical Sensitivity for Cancer. *Cancer Res* 1991;51:222–6.

11. Lilja H, Christensson A, Dahlén U, *et al.* Prostate-Specific Antigen in Serum Occurs Predominantly in Complex with α1-Antichymotrypsin. *Clin Chem* 1991;37:1618–25.

12. Christensson A, Björk T, Nilsson O, *et al.* Serum Prostate Specific Antigen Complexed to α1-Antichymotrypsin as an Indicator of Prostate Cancer. *J Urol* 1993;150:100–5.

13. Prestigiacomo AF, Lilja H, Pettersson K, *et al.* A Comparison of the Free Fraction of Serum Prostate Specific Antigen in Men with Benign and Cancerous Prostates: The Best Case Scenario. *J Urol* 1996;156:350–4.

14. Luderer AA, Chen Y-T, Soriano TF, *et al.* Measurement of the Proportion of Free to Total Prostate-Specific Antigen Improves Diagnostic Performance of Prostate-Specific Antigen in The Diagnostic Gray Zone of Total Prostate-Specific Antigen. *Urology* 1995;46:187–94.

15. Catalona WJ, Smith DS, Wolfert RL, *et al.* Evaluation of Percentage of Free Serum Prostate-Specific Antigen to Improve Specificity of Prostate Cancer Screening. *JAMA* 1995;274:1214–20.

16. Partin AW, Oesterling JE, Editors. The Clinical Usefulness of Percent Free Prostate-Specific Antigen. *Urology (Suppl. No. 6A)* 1996;48:1–87.

17. Vashi AR, Wojno KJ, Henricks W, *et al.* Determination of the "Reflex Range" and Appropriate Cutpoints for Percent Free Prostate-Specific Antigen in 413 Men Referred for Prostatic Evaluation Using the AxSYM System. *Urology* 1997;49:19–27.

18. National Committee for Clinical Laboratory Standards. *Primary Reference Preparations Used to Standardize Calibration of Immunochemical Assays for Serum Prostate Specific Antigen (PSA): Approved Guideline.* NCCLS Document I/LA 19-A. Wayne, PA: NCCLS, 1997.

19. Chen Z, Prestigiacomo A, Stamey TA. Purification and Characterization of a Prostate-Specific Antigen (PSA) Complexed to α1-Antichymotrypsin: Potential Reference Material for International Standardization of PSA Immunoassays. *Clin Chem* 1995;41:1273–82.

20. Stamey TA. Second Stanford Conference on International Standardization of Prostate-Specific Antigen Immunoassays: September 1 and 2, 1994. *Urology* 1995;45:173–84.

21. U.S. Department of Labor, Occupational Safety and Health Administration. 29 CRF Part 1910.1030, *Occupational Exposure to Bloodborne Pathogens; Final Rule. Federal Register* 1991;56(235):64175–82.

22. U.S. Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* HHS Publication (NIH) 93-8395. Washington: U.S. Government Printing Office. May, 1993:6–43.

23. World Health Organization. *Laboratory Biosafety Manual.* Geneva. World Health Organization, 1993:7–18;35–47;60–70;76–83.

24. National Committee for Clinical Laboratory Standards. *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue-Second Edition; Tentative Guideline 1991.* NCCLS Document M29-T2. Wayne, PA: NCCLS, 1991:1–43.

25. Woodrum D, French C, Shamel LB. Stability of Free Prostate-Specific Antigen in Serum Samples Under a Variety of Sample Collection and Sample Storage Conditions. *Urology (Suppl. 6A)* 1996;48:33–9.

26. Piironen T, Pettersson K, Suonpää M, *et al.* *In Vitro* Stability of Free Prostate-Specific Antigen (PSA) and Prostate-Specific Antigen (PSA) Complexed to α1-Antichymotrypsin in Blood Samples. *Urology (Suppl. 6A)* 1996;48:81–7.

27. Primus FJ, Kelley EA, Hansen HJ, *et al.* "Sandwich"-Type Immunoassay of Carcinoembryonic Antigen in Patients Receiving Murine Monoclonal Antibodies for Diagnosis and Therapy. *Clin Chem* 1988;34:261–4.

28. Schroff RW, Foon KA, Beatty SM, *et al.* Human Anti-Murine Immunoglobulin Responses in Patients Receiving Monoclonal Antibody Therapy. *Cancer Res* 1985;45:879–85.

29. Nahm MH, Hoffman JW. Heteroantibody: Phantom of the Immunoassay. *Clin. Chem.* 1990;36:829.

30. Boscato LM, Stuart MC. Heterophilic Antibodies: A Problem for All Immunoassays. *Clin Chem* 1988;34:27–33.

31. Chan DW, Bruzek DJ, Oesterling JE, *et al.* Prostate-Specific Antigen as a Marker for Prostatic Cancer: A Monoclonal and A Polyclonal Immunoassay Compared. *Clin Chem* 1987;33:1916–20.

32. Hortin GL, Bahnson RR, Daft M, *et al.* Differences in Values Obtained with 2 Assays of Prostate Specific Antigen. *J Urol* 1988;139:762–5.

33. Collins GN, Martin PJ, Wynn-Davies A, et al. The Effect of Digital Rectal Examination, Flexible Cystoscopy and Prostatic Biopsy on Free and Total Prostate Specific Antigen, and the Free-to-Total Prostate Specific Antigen, and the Free-to-Total Prostate Specific Antigen Ratio in Clinical Practice. *J Urol* 1997;157:1744–7.

34. Yuan JJJ, Coplen DE, Petros JA, *et al.* Effects of Rectal Examination, Prostatic Massage, Ultrasonography and Needle Biopsy on Serum Prostate Specific Antigen Levels. *J Urol* 1992;147:810–4.

35. Tchetgen M-B, Song JT, Strawderman M, *et al.* Ejaculation Increases the Serum Prostate-Specific Antigen Concentration. *Urology* 1996;47:511–6.

36. Stenman U-H, Leinonen J, Zhang W-M. Problems in the Determination of Prostate Specific Antigen. *Eur J Clin Chem Clin Biochem* 1996;34:735–40.

37. Morgan WR, Zincke H, Rainwater LM, *et al.* Prostate Specific Antigen Values after Radical Retropubic Prostatectomy for Adenocarcinoma of the Prostate: Impact of Adjuvant Treatment (Hormonal and Radiation). *J Urol* 1991;145:319–23.

38. Marley GM, Miller MC, Kattan MW, et al. Free and Complexed Prostate-Specific Antigen Serum Ratios to Predict Probability of Primary Prostate Cancer and Benign Prostatic Hyperplasia. *Urology 1996;48(6A):16-22.*

39. Vessella RL, Lange PH, Partin AW, et al. Probability of Prostate Cancer Detection Based on Results of a Multicenter Study Using the AxSYM Free and Total PSA Assays. *Urology* 2000;55: xx-xx.

40. Catalona WJ, Partin AW, Slawin KM, et al. Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease. *JAMA*; 1998; 279:1542-1547.

41. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Clinical Chemical Devices-Second Edition; Tentative Guideline.* NCCLS Document EP5-T2, March 1992.